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Allosteric modulation of G protein-coupled receptors

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Abstract

Allosteric modulation of G protein-coupled receptors is a relatively novel and unexplored pharmacological concept that may lead to more selective and more 'natural' drugs for these receptors. In particular, allosteric *enhancers* may serve as tools to intensify selectively a weakened hormone or neurotransmitter signal caused by a localized deficit, such as in Alzheimer's or Parkinson's disease. In this paper, attention is paid to the adenosine A_1 receptor, for which novel allosteric enhancers were synthesized and characterized that proved superior to the prototypic allosteric enhancer PD 81,723.

Keywords: Adenosine A_1 receptor; Allosteric modulation; G protein

1. Introduction

The mechanism of action for many drugs is often based on mimicking (as in the case of *agonists*) or blocking (as in the case of *antagonists* and *inverse agonists*) the action of an endogenous signaling molecule by competing at the same site (the *ligand binding site*) on a specific receptor. Recently, a new concept to interfere with drug action at G protein-coupled receptors (GPCRs) has emerged for some receptor subclasses, such as the muscarinic and the adenosine receptors. This *allosteric modulation* of the receptor by molecules binding at a second (*allosteric*) site is relatively unexplored yet for GPCRs, but quite common in the family of ion-channel receptors [1]. This indirect (allosteric) mechanism, i.e. the modulation of the efficacy or affinity of the endogenous ligand for its receptor, is the molecular basis of the therapeutic action of benzodiazepines that interact with $GABA_A$ ion-channel-coupled receptors. In contrast, there has been no therapeutic role found for directly acting agonists or antagonists on this receptor.

The potential benefits of allosteric drugs over agonists, antagonists and inverse agonists are substantial. It should be realized that, within the GPCR family, subtypes exist that bind the same hormone (or neurotransmitter) but have different tissue distributions, as well as functions. These receptor subtypes often have a high sequence homology, especially around the putative ligand binding site. Thus, in many cases the development of drugs that are not only highly selective for one receptor subtype, but have highly controlled effects on the function of that receptor and act in those tissues only where their action is desired, has proven difficult.

An allosteric drug, however, is thought to:

1. have no action when binding on its own to the receptor; it may modulate the actions of the naturally occurring hormone or neurotransmitter when the latter is released; the temporal aspects of the natural signaling mechanism may thus be preserved;
2. have a defined maximum effect that is determined by the cooperativity associated with its allosterism;
3. act selectively at various receptor subtypes by means of its own affinity, as well as its cooperativity;
4. be capable of selectively intensifying a weakened signal from a specific receptor subtype, if it has 'enhancing' properties. Such a drug may alleviate the effects caused by a localized neurotransmitter deficit, such as in Alzheimer's or Parkinson's disease.

Allosteric interactions on GPCRs have been observed for certain biogenic amine [2–8] and adenosine receptors [9]. For the purpose of this paper, we will focus only on one allosteric phenomenon, as well as on its

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potential for therapeutic exploitation: that on the adenosine A_1 receptor.

2. The adenosine A_1 receptor and its allosteric modulation

Extracellular adenosine is regarded as a local hormone that exerts numerous physiological actions in a variety of mammalian tissues. Its target, among others, is G protein-coupled adenosine receptors, subclassified as A_1, A_{2A}, A_{2B} and A_3 [10]. The adenosine A_1 receptor is coupled to a G_i protein, leading to a reduction in intracellular cAMP levels upon activation. This receptor is highly and widely expressed in the CNS, but is also present in other tissues, such as fat cells, bladder and heart [10–12]. A variety of adenosine-mediated effects (hypotension, inhibition of lipolysis, analgesia) occur via the adenosine A_1 receptor, rendering it an important target for pharmacological intervention. The wide distribution of adenosine receptors, however, poses some restrictions [10,13]. As an example, A_1 receptor agonists acting on fat cells reduce free-fatty-acid levels in the blood. This leads to a sensitization of insulin's action [14], which may be a very useful feature in non-insulin-dependent diabetes mellitus (type II diabetes). However, serious side effects may occur by the concomitant bradycardia and drop in mean arterial pressure due to interference with cardiovascular adenosine A_1 receptors [15]. Various strategies have been followed to circumvent all or some of these problems, such as the development of partial agonists for that purpose [16–18]. It was shown that some of these compounds were virtually 'silent' on the heart, while keeping a pronounced, full effect on adipose tissue [19].

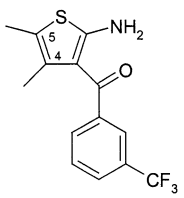


Fig. 1. Structure of PD 81,723 [(2-amino-4,5-dimethyl-thienyl)[3-(trifluoromethyl)phenyl]methanone].

Table 1
Apparent affinities (K_i values) of agonists in the absence or presence of PD 81,723 (10 μM) on the rat A_1 receptor

Compound	K_i (nM)	K_i (nM) + PD 81,723	Shift
CPA	4.0	2.0	2.0
R-PIA	7.4	4.0	1.9
2-Cl-Ado	39	23	1.7
NECA	50	39	1.3

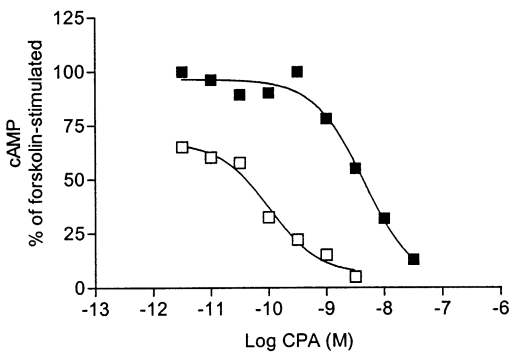


Fig. 2. Inhibition of forskolin-stimulated cAMP production in CHO-A_1 cells by CPA in the absence (■) or presence (□) of 10 μM PD81,723.

Another interesting approach is to enhance locally the action of adenosine itself, rather than 'replace' it by adenosine receptor agonists. This may sound utopic, but in 1990 Bruns and coworkers reported on various 2-amino-3-benzoylthiophene derivatives capable of enhancing the binding and activity of reference A_1 receptor agonists, such as N^6-cyclopentyladenosine (CPA) [9,20]. One of these 'allosteric modulators', PD 81,723 (2 - amino - 4,5 - dimethyl - thienyl)[3 - (trifluoromethyl)-phenyl]-methanone (Fig. 1), has been investigated pharmacologically in greater detail by various independent research groups [21–24].

PD 81,723 enhances up to twofold the binding of agonists such as CPA, R-PIA (N^6-*R*-phenylisopropyladenosine) or NECA (5′-*N*-ethylcarboxamidoadenosine) to adenosine A_1 receptors (Table 1, see also Ref. [25]). In displacement experiments of the radiolabeled antagonist [^{3}H]DPCPX from the rat adenosine A_1 receptor, the binding curve of CPA in the presence of PD 81,723 is shifted leftwards; it seems that CPA binds more efficiently, as lower concentrations of this agonist are needed to displace the same concentration of radioligand.

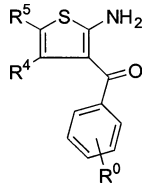
This 'enhanced' activity of CPA is also maintained in second messenger assays, in which lower concentrations of CPA (in the presence of PD 81,723) are needed for the inhibition of forskolin-stimulated cAMP production in cells expressing human adenosine A_1 receptors (Fig. 2). From Fig. 2 it is also evident — as was found in binding studies — that PD 81,723 has some antagonistic activity too.

The apparent reason for this is that PD 81,723 slows down the kinetics (dissociation) of ^{3}H-labeled agonists such as [^{3}H]CCPA from the receptor (data not shown); the half-life of 17 min for the dissociation of [^{3}H]CCPA alone from the rat A_1 receptor is increased to 25 min in the presence of 10 μM PD 81,723.

3. Synthesis of novel allosteric enhancers for the adenosine A_1 receptor

Recently, we have developed a series of novel PD 81,723 analogues. The synthesis of these derivatives is

Table 2
Structure, enhancing and antagonistic activity of 2-amino-3-benzoylthiophene analogs



Compound	R^0	R^4	R^5	Enhancement[a] (%)	Antagonism[b] (%)
PD81,723	3-CF_3	CH_3	CH_3	100	39 (±4)
1a	H	CH_3	CH_3	8 (±5)	14 (±3)
1b	3-Cl	CH_3	CH_3	80 (±19)	19 (±4)
1c	4-Cl	CH_3	CH_3	93 (±32)	41 (±6)
1d	H	CH_2CH_3	CH_3	31 (±4)	13 (±3)
1e	3-CF_3	CH_2CH_3	CH_3	112 (±10)	5 (±11)
1f	3-Cl	CH_2CH_3	CH_3	30 (±7)	22 (±2)
1g	4-Cl	CH_2CH_3	CH_3	97 (±25)	20 (±12)
1h	H	$-(CH_2)_4-$		47 (±4)	35 (±6)
1i	2-Cl	$-(CH_2)_4-$		73 (±19)	35 (±3)
1j	3-CF_3	$-(CH_2)_4-$		122 (±19)	32 (±8)
1k	3-Cl	$-(CH_2)_4-$		93 (±6)	51 (±5)
1l	3-I	$-(CH_2)_4-$		113 (±18)	66 (±1)
1m	4-CF_3	$-(CH_2)_4-$		131 (±11)	57 (±4)
1n	4-Cl	$-(CH_2)_4-$		123 (±15)	40 (±5)
1o	4-Br	$-(CH_2)_4-$		128 (±18)	42 (±4)
1p	4-I	$-(CH_2)_4-$		155 (±21)	64 (±8)
1q	4-NO_2	$-(CH_2)_4-$		34 (±22)	19 (±2)
1r	4-CH_3	$-(CH_2)_4-$		137 (±21)	30 (±3)
1s	4-CO_2CH_3	$-(CH_2)_4-$		44 (±9)	29 (n = 1)
1t	4-CO_2H	$-(CH_2)_4-$		29 (±3)	nd
1u	3,4-Cl	$-(CH_2)_4-$		151 (±24)	35 (±4)

[a] Enhancing activity (at 10 μM of test compound) is expressed as a percentage decrease (±SEM) in [^{3}H]CCPA dissociation over control (0%) and that of PD81,723 (100%, $n = 3$).

[b] Antagonistic activity is expressed as a percentage displacement (±SEM) of 0.4 nM of [^{3}H]DPCPX by 10 μM of test compound; nd: not determined.

relatively straightforward (see Refs. [26,27] and references cited therein). The 4,5-dimethyl group and the benzoyl moiety were targets for further modifications, leading to a series of, among others, 4,5-dialkyl (**1a**–**g**) and tetrahydrobenzo (**1h**–**u**) derivatives (Table 2). These derivatives were evaluated both as allosteric enhancers of agonist binding to the rat adenosine A_1 receptor, and as antagonists on this receptor. Among them, a number of compounds, in particular **1b**, **1j**, **1n** and **1u**, proved similar or superior to PD 81,723 in both enhancing activity and diminished antagonistic behavior.

4. Conclusion

The possibility of allosterically modulating receptors offers a novel pharmacological means of 'fine-tuning' receptor function. Whether this is a general feature of all or only of a subset of GPCRs remains to be established. Finally, elucidation of the molecular mechanisms of the allosteric interactions will provide useful insights that may help in the therapeutic exploitation of this phenomenon through the design and development of appropriate allosteric ligands.

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